Online Methods

Mice
C57BL/6 and SCID mice were obtained from the breeding facility of the Stanford University Veterinary Service Center. *Gsk3b*$^{+/-}$ mice (provided by G. R. Crabtree with permission from J. R. Woodgett) were maintained on a CD1 genetic background. All experiments on mice were performed with the approval and in accordance with Stanford's Administrative Panel on Laboratory Animal Care.

Inhibitors
The GSK3 inhibitors SB216763 (Sigma), GSK3-IX and alsterpaullone (EMDbiosciences) were dissolved in dimethylsulphoxide and used at the indicated concentrations. All other inhibitors (EMDbiosciences) were dissolved in dimethylsulphoxide and used at the concentrations indicated in Supplementary Table 1.

Cell cultures
All human leukaemia cell lines (Supplementary Table 2) were maintained in R10 medium (RPMI1640 supplemented with 10% FBS, 1% l-glutamine and penicillin/streptomycin). Immortalized mouse myeloid cells were maintained in R20/20 medium (RPMI1640 with 20% FCS, 20% WEHI-conditioned medium, l% l-glutamine and penicillin/streptomycin). Immortalized mouse B cells were cultured in OP9 medium (MEMα+GlutaMax 1 with 10% FBS, 1% l-glutamine, penicillin/streptomycin and 2μM β-mercaptoethanol) containing 1 ng ml^{-1} IL-7 when necessary. All culture medium was obtained from Gibco.

DNA constructs and virus production
Retroviral constructs (MSCV vector) encoding *MLL–ENL*, *MLL–LAF4*, *MLL–AF6*, *MLL–GAS7*, *E2A–PBX1*, *NUP98–HOXA9* and *E2A–HLF* were reported previously[21, 28, 37, 38, 39, 40]. Retroviral constructs encoding *CA–AKT*, *ER–CA–AKT*[41], and wild-type or S9A mutant GSK3B were constructed by cloning the respective complementary DNAs into MSCV using standard cloning techniques. Retrovirus production was performed as described previously[42]. Oligonucleotides for specific shRNA knockdown of *Gsk3a*, *Gsk3b* or *p27*^{Kip1} (sequences in Supplementary Table 3) were designed using PSICOLIGOMAKER 1.5 software (http://web.mit.edu/jacks-lab/protocols_table.html), and cloned into pSicoR-Puro or pSicoR-Hygromycin lentiviral vectors. Lentiviral stocks were produced as described previously[43].

Murine progenitor transformation assays
Myeloid progenitor transformation was performed as described previously[21] with minor modifications. In brief, c-Kit^{+} cells were isolated from the bone marrow of 4–8-week-old C57BL/6 mice or E16 fetal livers (*Gsk3b*$^{-/-}$ mice) using an auto-MACS and anti-c-Kit beads (Miltenyi Biotech). The c-Kit^{+} cells were spinoculated with retroviral supernatant in the presence of 5μg ml^{-1} polybrene for 2h at 1,350*g* and at 32°C. After spinoculation and after

overnight culture, cells were plated in methylcellulose medium (M3231; Stem Cell Technologies) containing 20 ng ml^{-1} stem cell factor, 10 ng ml^{-1} IL-6, granulocyte–macrophage colony-stimulating factor (GM–CSF), and IL-3 (R&D Systems) with appropriate antibiotic selection. After 5–7 days of culture, colonies were counted, pooled, and then 10^4 cells were replated in the same medium but without antibiotic. At the end of the fourth round, cells were transferred to R20/20 medium to establish continuous cell lines.
B cell progenitors were transduced as described previously[37] with minor modifications. Transduced cells were co-cultured on neo-resistant-irradiated OP9 stromal cells. After continuous passage and adaptation to liquid culture, immortalized cell lines were used for injections of syngeneic recipient mice. *MLL–AF4* B cell precursor leukaemia cell lines were generated by explantation of splenocytes collected from leukaemic mice.

Transduction of immortalized mouse cells
Immortalized mouse cells (20,000) were transduced with retroviral or lentiviral constructs by spinoculation at 2,500*g* or 1,200*g* for 2 h at 32 °C. Transduced cells were then resuspended in 200 µl of R20/20 or OP9 medium and transferred to 96-well plates. After overnight incubation at 37 °C, myeloid cells were plated in methycellulose medium containing IL-3, IL-6, GM-CSF and stem cell factor. Transduced B cells were plated in methycellulose medium containing IL-7.

Leukaemogenesis assays
Myeloid progenitors (wild type or *Gsk3b*$^{-/-}$) transformed by *MLL–ENL* were transduced with different lentiviruses, selected for drug resistance and then transplanted (10^6 cells) by intravenous injection into sub-lethally irradiated (2 Gy) C.B-17 *scid*/*scid* mice (6–8–weeks-old). For lithium treatment, irradiated (1 Gy) C57BL/6 mice were transplanted with *MLL–AF4*-transformed B cell progenitors (50,000 cells) and maintained on 0.4% lithium-carbonate-containing chow with saline water (Harlan Teklad). Lithium treatment was initiated 3 days before transplantation and continued for 30 days, at which point treatment was withheld for 5 days to allow recovery from drug-induced diuresis, and was then resumed. Development of acute leukaemia was confirmed by blood smear, peripheral blood leukocyte counts, FACS analyses and/or histology.

Flow cytometry
Staining of cells for FACS analysis was performed as previously described[42] using conjugated antibodies obtained from either BD Pharmingen or eBioscience. Cell cycle assays using propidium iodide staining, and apoptosis assays using annexin V staining, were performed as described[28]. BrdU incorporation was determined using the BrdU flow kit (BD Pharmingen) according to the manufacturer's instructions.

Cell proliferation and MTT assays
Cultured cells (10,000–20,000) were plated in 96-well plates in R10, R20/20

or OP9 medium (100 µl) containing different concentrations of the indicated kinase inhibitors (Supplementary Table 1) and incubated at 37 °C. The numbers of viable cells were determined by trypan-blue dye exclusion at the indicated times using a haemocytometer. For MTT assays, cells were cultured for 3–4 days and then quantified using a cell proliferation kit 1 under conditions recommended by the manufacturer (Roche).

Western blot

Cells were lysed in buffer A (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA) containing protease inhibitors (complete mini protease inhibitors; Roche). Proteins (40 µg) were subjected to SDS–PAGE and analysed by western blot using primary antibodies specific for GSK3 (Upstate Biotechnology), β-catenin (Upstate Biotechnology), phosph-GSK3 (Cell Signalling), AKT (Cell Signaling), tubulin (Sigma), p27 or p21 (Santa Cruz Biotechnology).